Synthesis of Nw-[18F]Fluoroacetyl-5-hydroxytryptamine from Ethyl p-Tosyloxyacetate

III. 2. Synthesis of N^{ω}-[^{18}F]Fluoroacetyl-5-hydroxytryptamine from Ethyl *p*-Tosyloxyacetate

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Introduction

5-Hydroxytryptamine (serotonin) (I) is found widely distributed in invertebrates and vertebrates.[1] In mammals it is found in the blood platelets, intestines, and in large quantities in the brain. In the latter it is localized in certain area rather than being uniformly distributed.[2] N^{ω}-acetyl-5-hydroxytryptamine (N^{ω}-acetylserotoin)(II) is an important precursor for the biosynthesis of N^{ω}-acetyl-5-methoxytryptamine (melatonin)(III), the pineal gland hormone. In a previous paper,[3] we reported the rapid synthesis of [*fluoroacetyl*-^{18}F]fluoromelatonin (IV), a potential diagnostic imaging agent, from [^{18}F]fluoride and ethyl *p*-tosyloxyacetate. We also reported the concise syntheses of [*carbonyl*-^{11}C]melatonin and N^{ω}-[*carbonyl*-^{11}C]acetylserotonin as imaging agents.[4] The introduction of ^{18}F (β^+ decay, $t_{1/2}$=110 min) at the terminal position of (II) is attractive for a diagnostic imaging agent in a positron emission tomography (PET) study.

As part of the investigation of the synthesis of positron emitting derivatives for PET study, we describe here the synthesis of N^{ω}-[^{18}F]fluoroacetyl-5-hydroxytryptamine (V), N^{ω}-[^{18}F]fluoroacetylserotonin, from ethyl *p*-tosyloxyacetate.

Results and discussion

Unlabelled N^{ω}-fluoroacetyl-5-hydroxytryptamine (VI) was prepared from (I) with fluoroacetic acid by the ordinary method using dicyclohexylcarbodiimide (DCC). The yield of (VI) based on (I) was 71.5%.

[^{18}F]Fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction from a circulating 20%-enriched [^{18}O]water target using the Tohoku University Cyclotron.[5] The ^{18}F nuclide thereby formed was converted to potassium [^{18}F]fluoride with potassium carbonate. After addition of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan (Kryptofix 222), the resulting mixture was evaporated to dryness. The residue and ethyl *p*-tosyloxyacetate (derived from ethyl bromoacetate and silver *p*-toluenesulfonate) in anhydrous acetonitrile

were heated at 82 °C for 10 min with stirring to give ethyl [^{18}F]fluoroacetate. The ester was then hydrolyzed with alkali and condensed with (I) in the presence of DCC to afford desired compound (V) in a 13.5% radiochemical yield. The synthesis time, radiochemical purity, and the specific activity (end of bombardment) are *ca.* 90 min, >98%, and 600 mCi/μmol, respectively. The synthetic pathways of (V) and its radio-preparative high performance liquid chromatogram (HPLC) are shown in Figs. 1 and 2, respectively.

The medical use of (V) as a diagnostic imaging agent is being investigated and the result will be reported elsewhere.

Experimental

Silica gel 60, Kryptofix 222, Extrelut-3 columns, and TLC plates were purchased from E. Merck AG, Ger. Silver *p*-toluenesulfonate was from Sigma Chem. Co., USA. Ethyl bromoacetate was from Wako Chem. Ltd. Japan and distilled under reduced pressure. The other reagents were obtained commercially (Wako) and used without further purification. HPLC analyses were carried out either with a Waters Assoc. USA model 6000 equipped with a UV(254 nm) detector and a refractive index detector or with a Waters Assoc. model 4500 equipped with a UV detector and a radioactivity monitor. The packed columns [Cica-Merck Hiber Lichrosorb RP-8-7μm (Kanto Chem. Co. Inc. Japan) and YMC-Pack A-324 (Yamamura Chem. Lab. Co. Japan)] were used in HPLC. Absorption and ^{1}H NMR(90 MHz) spectra were recorded with a Hitachi Japan model U-3210 spectrophotometer and a JEOL Japan model FX9OQ spectrometer, respectively. TLC analysis was carried out over a pre-coated silica gel 60F_{254} plate and its mobile phase was dichloromethane/ethanol (9/1, v/v). The detection was made with a UV lamp.

N$^{\omega}$-Fluoroacetyl-5-hydroxytryptamine (VI).

To a suspension of 5-hydroxytryptamine (I) hydrochloride (2.56 g, 12 mmol) in tetrahydrofuran (THF)(300 ml), was added sodium fluoroacetate (1.8 g, 18 mmol) in water(12 ml) and 3 N hydrochloric acid (3 ml). DCC (5.15 g, 25 mmol) in THF (25 ml) was added to the resulting clear solution. The mixture was stirred overnight at room temperature and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was dissolved in a mixture of dichloromethane and ethanol (9/1, v/v). The resulting solution was chromatographed over a silica gel column (3×20 cm) to give oil of (VI) (2.01 g) in a 71.5% yield. The oil is further purified by the use of preparative HPLC to give colorless glassy mass of (VI).

Anal. Found: C, 60.84; H, 5.64; N, 11.96%. Calcd. for $C_{12}H_{13}FN_2O_2$: C 61.01; H, 5.55; N, 11.86%. UV(CH_3OH): λ_{max} nm (log ε) 222 (4.38), 278 (3.79), and 300 (3.66). ^{1}H NMR[$CDCl_3$, internal $(CH_3)_4Si$]: δ 1.71 (lH, broad s, -OH), 2.94 (2H, t, J=7.0 Hz, side chain), 3.69 (2H, t, J=7.0 Hz, side chain), 4.78 (2H, d, J_{HF}=47.4 Hz, -CH_2F), 6.44 (lH, broad s, =NH), 6.80 (lH, dd, $J_{6,7}$=8.5 Hz, $J_{4,6}$=2.2 Hz, H-6), 7.02 (lH, d, $J_{4,6}$=2.2 Hz,

H-4), 7.23 (lH, d, $J_{6,7}$=8.5 Hz, H-7), 7.26 (lH, s, H-2), and 7.96 (lH, broad s, =NH). TLC: R_f 0.58.

N^{ω}-[^{18}F]Fluoroacetyl-5-hydroxytryptamine (V).

[^{18}F]Fluoride was produced from the proton bombardment of 20% enriched [^{18}O]water.[5] To the aqueous solution of [^{18}F]fluoride, was added a mixture of aqueous potassium carbonate (33 μmol/0.2 ml) and Kryptofix 222 (27 mg, 72 μmol). The resulting solution was dried at 90 °C in a stream of dry nitrogen gas. To the residue, a solution of ethyl *p*-tosyloxyacetate (5.4 mg, 21 μmol) in acetonitrile (1 ml) was added. The mixture was heated at 82 °C for 10 min with stirring and cooled. After addition of 1 N aqueous potassium hydroxide (0.5 ml), the reaction mixture was heated for an additional 10 min and acidified with 2 N hydrochloric acid (1 ml). The mixture was then charged on an Extrelut-3 column and eluted with ethyl ether. The effluent was evaporated to dryness and the residue was added to a mixture of (I) hydrochloride (5.3 mg, 25 μmol) in acetonitrile (1 ml) and DCC (103 mg, 0.15 mmol) in acetonitrile (1 ml). The mixture was heated at 82 °C for 10 min with stirring and cooled. After addition of water (2 ml), the mixture was filtered. The filtrate was passed through an Extrelut-3 column and eluted with a mixture of dichloromethane and ethanol (25/1, v/v). The effluent was then passed through a Sep-Pak C_{18} cartridge (Waters Assoc. USA) and eluted with the same solvent. The eluting solution was evaporated to dryness under reduced pressure and the residue was dissolved in methanol/water (70/30, v/v)(0.5 ml). The solution was then subjected to preparative HPLC. The radiochromatogram is shown in Fig. 2. A radioactivity peak corresponding to (V) was then collected and the identity of the peak was confirmed by analytical HPLC (Column, mobile phase, flow rate and retention time are Hiber Lichrosorb RP-8-7μm (4.0×250 mm), CH_3CN/H_2O (60/40, v/v) 1.0 ml/min, and 2.88 min, respectively). The total synthesis time, the radiochemical yield and purity, and the specific activity (EOB) are *ca* .90 min, 13.5%, >98%, and 600 mCi/μmol, respectively.

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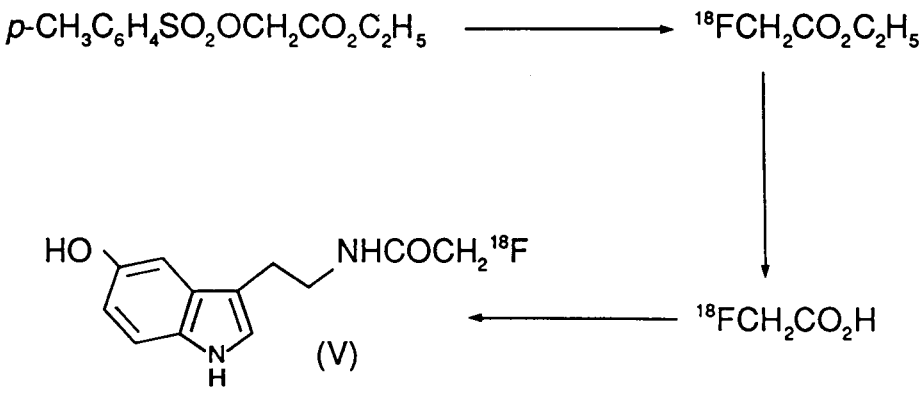
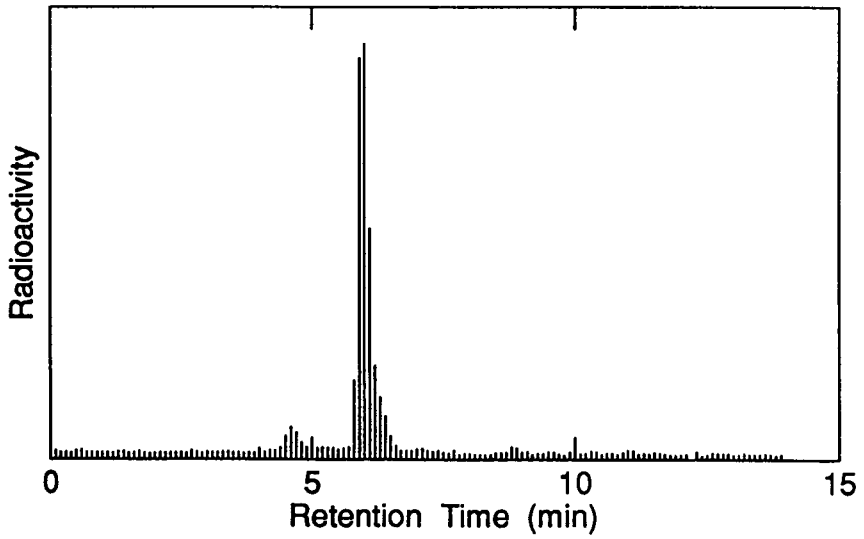


Fig. 1. Synthetic Pathway of (V) from Ethyl *p*-Tosyloxyacetate.



Column:	YMC-Pack A-324
Column Size:	10 x 300 mm
Mobile Phase:	CH_3OH/H_2O (70/30)
Flow Rate:	3.0 ml/min

Fig. 2. Radio-preparative HPLC Chromatogram of Reaction Mixture.
The large peak corresponds to the desired product (V).